NIEHS News

Orphan Receptors

A number of chemicals found in the environment exert their effects by interacting with specific receptor molecules. Receptors are cellular proteins with specific and high affinity binding for effector molecules (ligands). This binding results in a functional response and change. Nuclear hormone receptors recognize steroid hormones (estrogen, progesterone, androgen, etc.), thyroid hormones, vitamins, and retinoic acid. The hormone–receptor complex alters expression of key target genes and leads to functional alterations in cells, tissues, and organs. Understanding receptor binding and mechanism of action is key to interpreting signals from our internal as well as the external environment.

For example, a number of foreign chemicals, such as DDT and polycyclic chlorinated biphenyls, have been reported to interact with the estrogen receptor, eliciting many of the same cellular and physiological effects of the natural female sex hormone estradiol. Although the hormonal activity of these xenobiotic estrogens has been clearly demonstrated, their effects on human health is less clear. Chemicals need to be screened for their potential to interact with nuclear hormone receptors, which in turn may help explain their toxic actions.

Research in the area of receptor-mediated mechanisms is expanding at NIEHS. Cary Weinberger has joined Kenneth Korach's research section at NIEHS to supervise research on orphan receptors. Weinberger will also interact with existing groups at NIEHS that have been studying other receptor systems. These areas are headed by Korach, studying estrogen receptors and estrogen hormone action; Anton Jetten, investigating retinoids and retinoic acid receptors; and George Lucier, studying TCDD and dioxin receptors.

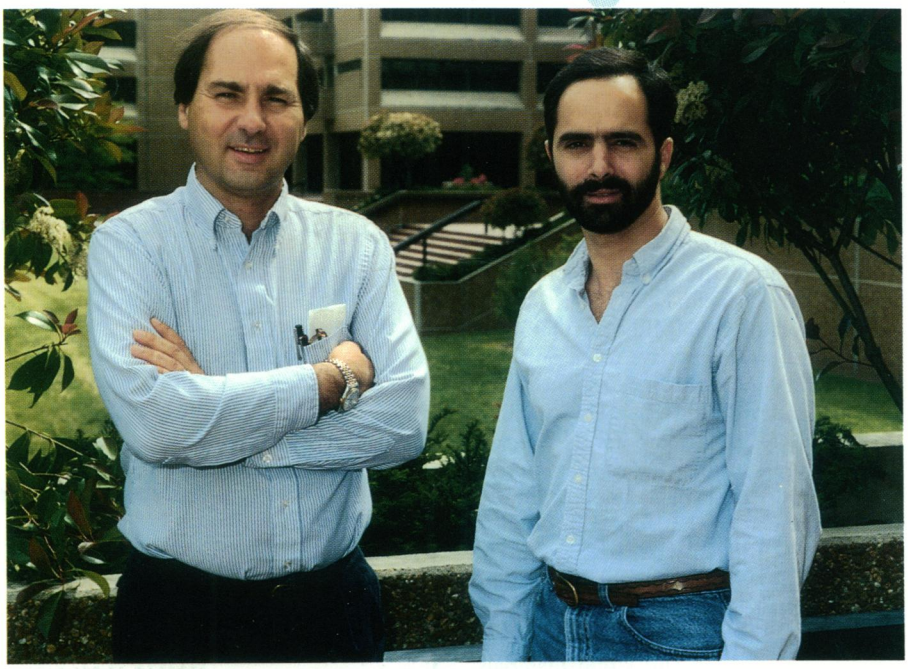
Weinberger comes to NIEHS from a Ligand Pharmaceuticals in San Diego, California. Prior to his work at Ligand Pharmaceuticals, he spent a year at NIH and did postdoctoral training at the Salk Institute in Ronald Evans's laboratory. During that time, Weinberger's studies were highlighted by the initial cloning and expression of the human glucocorticoid receptor gene, description of the relationship of the human GR to the V-*erb*-A oncogene product, and the identification and expression of novel thyroid receptors.

Identification and characterization of the nuclear hormone receptor family of proteins began several years ago with the initial cloning of the estrogen and glucocorticoid hormone receptors. Since that time, other members of this family were identified for which there are no known ligands and thus were called "orphan receptors." The role of orphan receptors in environmental disease is a promising area. Further characterization of orphan receptors and their endogenous ligands will provide a crucial link to understanding the molecular mechanisms through which exogenous chemicals exert toxic effects and through which natural substances influence physiologic processes.

Low-stringency hybridization has added about 25 orphan receptors to the list of known nuclear receptors. Molecular techniques that enable researchers to switch functional domains of the proteins offers a conspicuous means for identifying the ligands binding to each orphan receptor. Construction of hybrid receptors, for example, containing the glucocorticoid-receptor DNA binding domain linked to an orphan receptor's putative ligand binding domain identified the first retinoic acid receptor (RAR) gene. Cary Weinberger of NIEHS plans to use this approach to investigate specific classes of environmental chemicals.

Identification of the orphan receptor ligands is falling far behind receptor gene isolations and has directly challenged the resourcefulness of researchers. Determining an orphan receptor's ligand dependency remains the rate-limiting step for further characterizing functional aspects of receptors. However, rapid tests of response-element binding offer some insights into the assembly of transactivation assays. Some of the newest members of the orphan receptor class underscore the excitement and perplexities of the initial discovery of receptor ligand-dependency. Two new subfamilies include four members of the peroxisome proliferator-activated receptors (PPAR) as well as three of the so-called retinoid X receptor (RXR) kindred.

PPAR was initially characterized as being activated by classes of xenobiotics including plasticizers and lipid-lowering drugs. At least two of the four-member PPAR subfamily can be activated by clofibric acid, nafenopin, and a Wyeth-Ayerest compound called 14,643 with potencies in the supramicromolar range. These agents induce acyl coA oxidase, which is responsible for fatty acid β-oxidation, but their qualifications to lower triglyceride and cholesterol levels are complicated by their possible hepatocarcinogenicity. Endogenously circulating unsaturated fatty acids including oleic and linoleic acids were also found to activate PPAR, while another study has shown that the synthetic arachidonate 5,8,11,14-eicosatetraynoic acid is about 50 times more potent for PPAR than the Wyeth compound. Identification of exogenous and endogenous ligand activators will require researchers to plan and bridge molecular techniques and physiological research approaches for success.



Investigating orphan receptors. Kenneth Korach (left) and Cary Weinberger.

Staff of the National Clearinghouse for Worker Safety and Health Training surround a sculpture of labor leader George Meany (left to right): Katherine Roberts, Jeffrey MacDonald, Betsy Lewis, Joyce Reimherr.

NIH has recently launched a new initiative to investigate bionutrition. Weinberger has a strong interest in studying the mechanistic action of nutritional agents such as vitamins. Successful correlations of RAR and RXR with the vitamin A metabolites all-*trans*-retinoic acid and 9-*cis*-retinoic acid have redirected thinking about molecules such as vitamin E, whose functions have been attributed solely to its antioxidant properties. The antioxidant properties of vitamin E or α-tocopherol are especially interesting considering that this molecule is a terpene structurally similar to vitamin A.

One of the classical features of vitamin E deficiency is the inability of rodents to maintain pregnancies. Vitamin E-deficient rodents typically spontaneously resorb 15-day-old fetuses due to suppressed development of mesodermal tissues including the blood islands of the yolk sac and embryonic liver. Other cardinal deficiency signs include inductions of catabolic lysosomal enzymes that produce a muscle wasting from cellular protein and nucleic acid breakdown. These physiological changes may result from the vitamin's actions as a receptor co-activator, perhaps operating like RXR only in the presence of other *trans*-acting factors to promote gene transcription. The presence of vitamin E-binding polypeptides in liver cytoplasm has been established, although the biochemical evidence was relatively weak. These polypeptides may be more akin to the cellular retinol-binding proteins, which are thought to function more in a transport role for vitamin A. If vitamin E operates via an identified or unrecognized orphan receptor species, it most likely will require cell culture system for analysis. Such observations, considered with the multiple roles of vitamin A metabolites, may outline future studies of the role of vitamin E and other nutritional factors and environmental chemicals as receptor-transducing signals.

National Worker Training Clearinghouse

The NIEHS Superfund Worker Training Program has awarded a new two-year contract to the George Meany Center for Labor Studies in Silver Spring, Maryland, to operate the National Clearinghouse for Worker Safety and Health Training for Hazardous Materials, Waste Operations and Emergency Response. The clearinghouse will support the nationwide NIEHS training program, facilitating the transmission of technical information and curricula developed for safety and health training programs for hazardous waste and emergency personnel.

NIEHS was given major responsibility for initiating a training grants program under the Superfund Amendments and Reauthorization Act of 1986 (SARA). The major objective of this program is to fund nonprofit organizations in developing and delivering training to workers who handle hazardous wastes or who respond to accidental releases of hazardous materials.

Although NIEHS has developed a solid national training program for workers in high-risk occupations, the safety and health problems at toxic waste cleanup sites have substantially increased in extent and severity. After initial delays in beginning remediation at waste sites, the EPA Superfund program has been supplemented with even larger environmental restoration programs by the Departments of Energy and Defense.

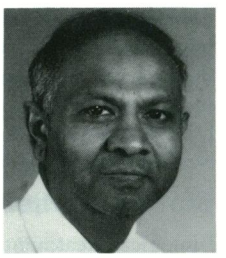
In addition to creating a resource library that holds all the curricula created by the NIEHS training program, the clearinghouse at the George Meany Center publishes a monthly news brief and activity report including information about hazardous materials, hazardous wastes and emergency response, and regulatory progress. The clearinghouse also arranges and manages technical workshops related to scientific, administrative, and regulatory issues associated with training for hazardous waste workers and emergency responders.

The George Meany Center for Labor Studies is a residential adult-learning center that provides leadership and technical education for the members, staff, and officers of national and international unions affiliated with the AFL-CIO. The Meany Center is also home to the Railway Workers Hazardous Materials Training Program, which is one of the eighteen cooperative agreement awardees that is supported through the NIEHS Worker Training Program. Further information on the clearinghouse or the NIEHS Worker Training Program can be obtained by calling (301) 431-5425.

Carcinogenesis and Diet Restriction

Rodents are the most commonly used animal models for chronic toxicity and carcinogenicity studies. The National Toxicology Program has typically used two-year studies with both sexes of rats and mice to evaluate the carcinogenic potential of chemicals.

In recent years, the survival of many strains of rats at the end of two-year studies has been less than 40%. This decreased survival is a serious concern to researchers involved in evaluating the safety and carcinogenic potential of drugs, food additives, pesticides, and other chemicals.



G. N. Rao

Diet is one of the most important environmental factors that influence survival. Diet restriction of greater than 30% for rats not subjected to chemical treatment lowers body weight, lowers the incidences of body weight-associated tumors, and increases survival at the end of two-year studies. Diet restriction markedly decreases incidences of tumors induced by chemicals and makes the animal irritable and aggressive. Furthermore, chemicals influence energy utilization, and diet restriction may disproportionately influ-